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EVOLUTION OF BUCCAL APPARATUS AND DIET RADIATION IN THE SACOGLOSSA (OPISTHOBRANCHIA) (**)

Abstract

The Sacoglossa are suctorial feeders, and the majority of species are stenophagous herbivores, feeding on the cell sap of marine plants. In the Sacoglossa evolution appears to be closely associated with diet radiation. All shelled Sacoglossa feed on the morphologically variable algal genus *Caulerpa*.

Changes in diet has occurred in parallel in the major groups of shell-less Sacoglossa. Pharynx musculature and radular teeth of several species of Sacoglossa are described. Based on this and previous studies, character analysis is performed, and the most important morphological and functional «innovations» are identified. These are: muscular suspension of ascending limb of radula, transverse muscles forming a functional separation of ascending and descending limbs of radula, blade-shaped teeth with median denticles, sabot-shaped teeth, and shift in importance from ascending to descending limb of radula. Radula suspending muscles are found in all sacoglossans, transverse muscles in the shell-less Sacoglossa. Blade-shaped teeth have evolved 2 or 3 times within the major lines of evolution, sabot-shaped teeth only in the stiligeroid line. The functional shift apparently occurred rather late, after the loss of the shell and pharyngeal pouches.

Riassunto

I sacoglossi sono opistobranchi erbivori che si alimentano succhiando il contenuto cellulare di diverse piante marine. La maggior parte di essi è stenofaga. L'evoluzione del gruppo sembra essere strettamente collegata alle loro scelte alimentari. Tutti i sacoglossi conchigliati si alimentano di varie alghe appartenenti al genere *Caulerpa*, mentre tra le specie non conchigliate, parallelamente alla loro evoluzione, è avvenuta una forte specializzazione della dieta.

Viene qui descritta la muscolatura faringea ed i denti radulari di diverse specie di sacoglossi. Grazie a questo ed altri studi, è stato possibile condurre un'analisi dei caratteri, identificando le più importanti «innovazioni» morfologiche e funzionali. Queste sono: la sospensione muscolare del ramo ascendente della radula, i muscoli trasversali che formano una separazione funzionale fra il ramo ascendente e discendente della radula, i denti a forma di lama con denticoli mediani, i denti a forma di zoccolo olandese o cucchiaio (sabot), e la perdita d'importanza del ramo ascendente nei confronti di quello discendente. Muscoli sospensori della radula si rinvengono in tutti i sacoglossi, mentre i muscoli transversali sono presenti solo nei sacoglossi non conchigliati. I denti a forma di lama hanno subito almeno 2-3 cambiamenti evolutivi nell'ambito delle maggiori linee filogenetiche dei sacoglossi, mentre quelli a cucchiaio appaiono solo all'interno del gruppo degli Stiligeroidi.

Il cambiamento funzionale della radula è apparentemente avvenuto piuttosto tardi, dopo la perdita della conchiglia e delle tasche faringee.

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Introduction

The Sacoglossa are suctorial feeders, and the majority of species are stenophagous herbivores, feeding on the cell sap of marine plants. The buccal apparatus in the Sacoglossa consists of the oral tube, the muscular pharynx, and the radula (Fig. 1). The oral tube of most sacoglossans is short and non-muscular. The sacoglossan pharynx and radular teeth show several autapomorphies to distinguish them from other opisthobranchs (JENSEN, 1991, in press).
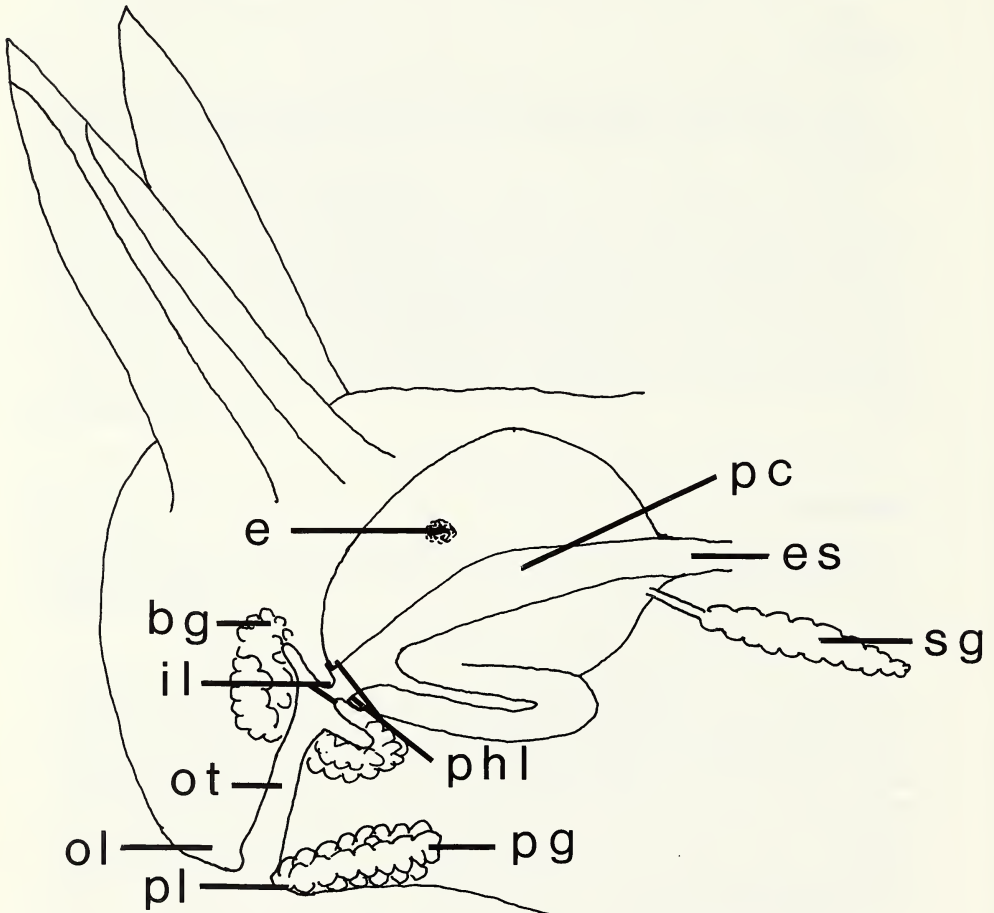

Figure 1. Schematic drawing of buccal apparatus in a sacoglossan.
Legend: **bg** - buccal glands; **e** - eye; **es** - oesophagus; **il** - inner lips; **ol** - outer lips; **ot** - oral tube; **pc** - pharyngeal cavity; **pg** - pedal glands; **phl** - pharyngeal lips; **pl** - pedal lobe; **sg** - salivary gland.

The suctorial pharynx of sacoglossans is composed of 4 muscular units: (1) the dorsal septate muscle, (2) the odontophore, (3) the ventral, longitudinal ascus-muscle, and (4) the pharyngeal pouch. The odontophore contains 2 or 3 functionally distinct groups of musculature: (1) the odontophore muscles (mainly dorso-ventral), (2) the radula suspending muscles (fan-shaped), and (3) the ventral, transverse muscles forming a functional separation between the ascending and descending limbs of the radula (JENSEN, in press).

The sacoglossan radula is uniseriate and composed of (1) an ascending limb completely enclosed within the odontophore musculature, (2) a descending limb of about equal length, surrounded by the ascus-muscle, and (3) an ascus in which old, used teeth are accumulated throughout life, either rolled up into a spiral, or in a densely packed heap (JENSEN, 1991).

Parallel evolution is very common in the opisthobranchs (GOSLINER & GHISELIN, 1984). In the Sacoglossa evolution appears to be closely associated with diet radiation (JENSEN, 1993a in press). All shelled Sacoglossa feed on the morphologically variable algal genus *Caulerpa*. Changes in diet has occurred in parallel in the major groups of shell-less Sacoglossa. This has resulted in several parallelisms of the feeding apparatus (JENSEN, 1993a).

The Sacoglossa contains 3 distinct superfamilies: the shelled Conchoidea, the cerata-bearing Stiligeroidea, and the parapodia-bearing Elysioidea. Previous studies, mainly on the Elysiidae, have shown that all the major muscle groups of the sacoglossan pharynx have evolved differently in the different species (JENSEN & WELLS, 1990; JENSEN, 1992, in press). Also, correlations between shape of radular teeth and diets have been examined (JENSEN, 1993a). In the present study information on pharynx musculature and radular teeth of several species from the major evolutionary lines will be presented. Based on this information as well as that of the previous studies, character analysis is performed. For outgroup comparison the very well studied *Monodonta lineata* and *Philine aperta* have been used (FRETTER & GRAHAM, 1962; HURST, 1965). Functional aspects have been included in the analysis. Also, the parallelisms related to diet changes will be discussed.

Materials and methods

Collecting data for specimens used in the present study are listed in Table 1. Specimens were relaxed in 7% $MgCl_2.6H_2O$ mixed with seawater (1:1). They were fixed in neutral 4% formaldehyde and then transferred to 70% or 80% ethanol. For fine dissection animals were lightly stained with acetocarmine. Radular mounts for SEM were prepared as described in JENSEN (1992). Serial sections of *Caliphylla mediterranea* were generously given to the author by Dr. Tom Gascoigne.

Table 1. List of material used in present study. WA - Western Australia; USVI - U.S. Virgin Islands.

| Species	Location	Date	Collector
Ascobulla fischeri	Triggs Isl. WA	30 Mar. 69	S. Slack-Smith WAM186-88
	Albany, WA	Jan. 88	K.R. Jensen
Lobiger sagamiensis	Hong Kong	April 83	K.R. Jensen
Elysia flavomacula	Hong Kong	April 86	K.R. Jensen
Elysia cf. *maoria*	Rottnest Isl. WA	Jan. 91	K.R. Jensen
Caliphylla mediterranea	Livorno, Italy	June 78	T. Gascoigne
Cyerce antillensis	St. Thomas, USVI	April 82	K.R. Jensen
Hermaea cruciata	Florida	Jan. 80	K.R. Jensen
Aplysiopsis formosa	Azores	July 91	K.R. Jensen
Aplysiopsis smithi	California	July 86	K.R. Jensen
Costasiella pallida	Hong Kong	April 86	K.R. Jensen
Ercolania nigra	Denmark	Aug. 89	K.R. Jensen
Ercolania translucens	Rottnest Isl. WA	Feb 81	J.S. Bleakney
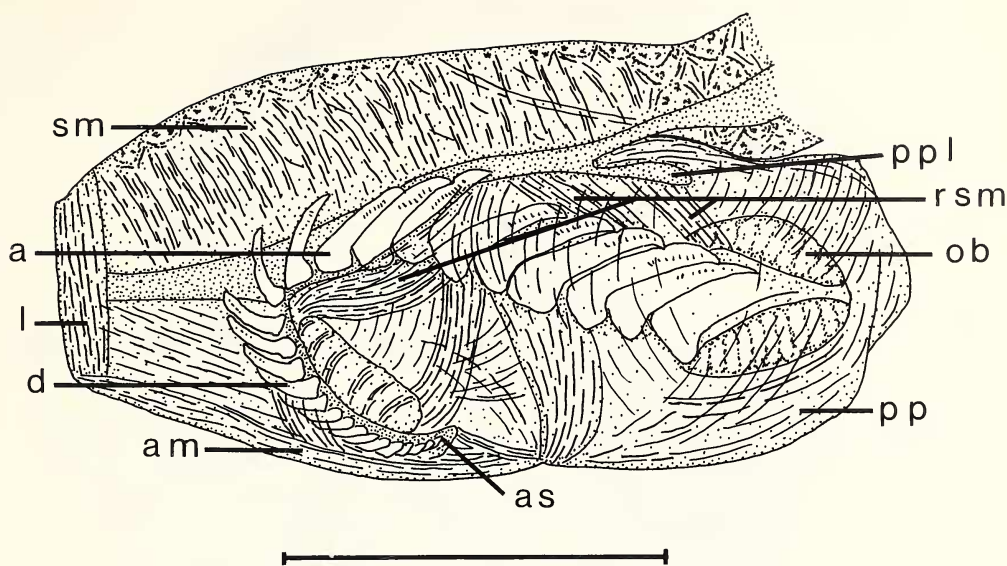
Figure 2. Sagittally sectioned pharynx of *Ascobulla fischeri* (WAM 186-88). Scale line = 0.5 mm. Legend: **a** - ascending limb; **am** - ascus-muscle; **as** - ascus; **d** - descending limb; **l** - pharyngeal lips; **ob** - odontoblasts; **pp** - pharyngeal pouch; **ppl** - lumen of pharyngeal pouch; **rsm** - radula suspending muscles; **sm** - dorsal septate muscle.

Results

PHARYNX

The pharynx of the shelled *Ascobulla fischeri* has a flat dorsal septate muscle, a thin ascus-muscle, and a short, collar-like pharyngeal pouch (Fig. 2). The odontophore is positioned rather far back in the pharynx. The ascending limb of the radula is much longer than the descending limb, and the posterior tip of the radular sac extends all the way to the bottom of the pharyngeal pouch. Three to 4 teeth of the ascending limb are visible on the dorsal surface of the odontophore. Only a few radula suspending muscles are located on the dorsal surface of the ascending limb. The most prominent radula suspending muscles are ventral of the ascending limb. The odontophore muscles form dense layers on either side of the radula and appear to be continuous with the muscles of the pharyngeal pouch. About 4 teeth of the descending limb are visible on the anterior free tip of the odontophore. The descending limb ends in a straight row which is located within the odontophore. Dorsal to the descending limb is a distinct muscular «cushion» apparently composed of dorsoventral muscles. Thus there are no transverse muscles separating the ascending and descending limbs of the radula. The posterior tip of the descending limb is connected to the ascending limb by a strong strand of radula suspending muscle. The lumen of the pharyngeal pouch is only a small pocket at the dorsal part of the pharyngeal pouch, just below the esophagus.
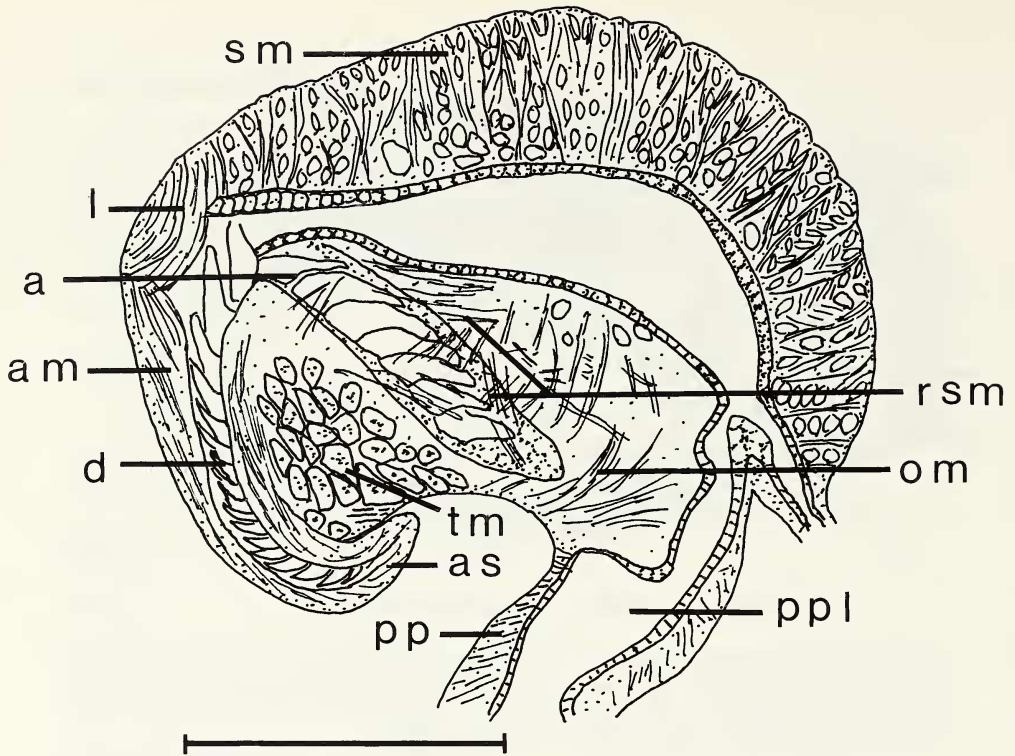
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Figure 3. Sagittal section through pharynx of *Caliphylla mediterranea*. Scale line = 0.5 mm. Legend: **a** - ascending limb; **am** - ascus-muscle; **as** - ascus; **d** - descending limb; **l** - pharyngeal lips; **om** - odontophore muscles; **pp** - pharyngeal pouch; **ppl** - lumen of pharyngeal pouch; **rsm** - radula suspending muscles; **sm** - dorsal septate muscle; **tm** - transverse muscles.

Sections of the pharynx of the polybranchiid *Caliphylla mediterranea* (Fig. 3) show that it has a thick dorsal septate muscle and a large pharyngeal pouch. The epithelium of the pharyngeal cavity is pigmented, as is that of the wide, paired lumina of the pharyngeal pouch. There is a distinct dorsal food groove. The ascending limb is rather far towards the ventral surface of the odontophore. There are 3-4 teeth on the free tip of the odontophore. The ascus-muscle is rather short and steeply inclined relative to the longitudinal axis of the pharynx. Its posterior tip is not attached to the ventral pharynx wall. Teeth in the ascus are rolled in a spiral. Most of the radula suspending muscles are dorsal to the ascending limb. A prominent muscle attaches to the anteriormost tooth in the ascending limb and runs along the anteriormost teeth in the descending limb. It continues along the ventral surface of the pharynx to a point behind the tip of the ascus-muscle. Dorsal to this muscle is a thick layer of transverse muscles forming a tall, narrow «stalk» separating the ascending and descending limbs of the radula. The pharyngeal pouch consists of mainly dorso-ventral muscles. The paired lumina of the pharyngeal pouch are narrow in the anterior part of the pharyngeal pouch («stalk») and wide in the posterior part. The pouch is somewhat twisted.
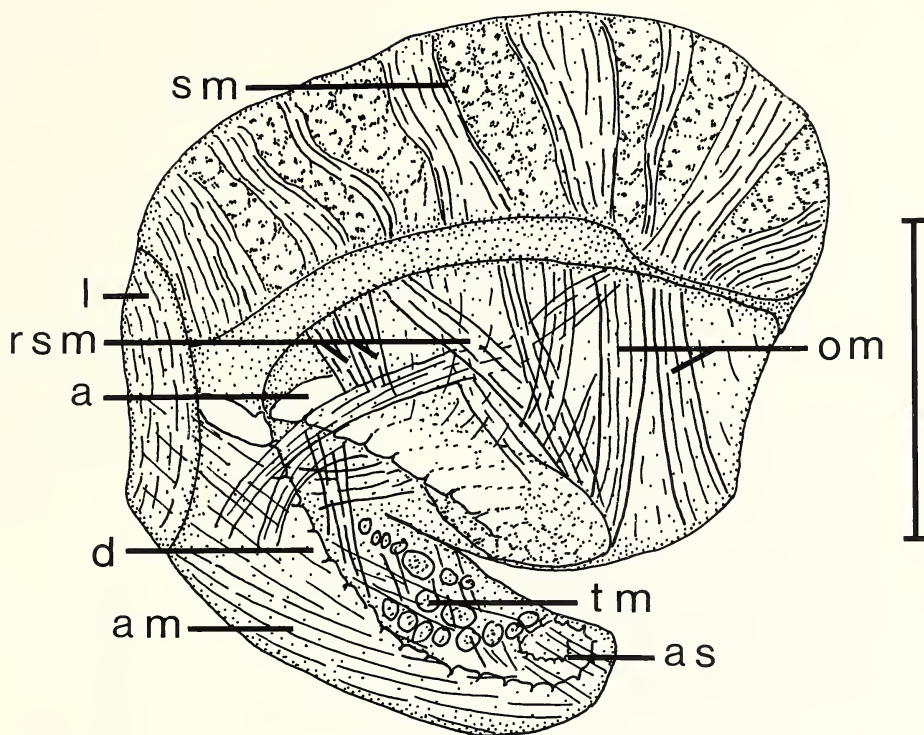
Figure 4. Sagittally sectioned pharynx of *Aplysiopsis formosa*. Scale line = 0.5 mm.
Legend: **a** - ascending limb; **am** - ascus-muscle; **as** - ascus; **d** - descending limb; **l** - pharyngeal lips; **om** - odontophore muscles; **rsm** - radula suspending muscles; **sm** - dorsal septate muscle; **tm** - transverse muscles.

The pharynx of *Aplysiopsis formosa* has a thick, distinctly septate dorsal wall (Fig. 4). The odontophore is large, and the ascending limb of the radula is located rather far towards the ventral surface. It does not reach the posterior end of the pharynx. Apparently there are few radula suspending muscles dorsal to the ascending limb. This probably indicates that back and forth movements of the ascending limb are not important. The odontophore muscles behind the ascending limb of the radula are prominente. There is a thick layer of transverse muscles separating the ascending and descending limbs. The longitudinal muscles of the ascus-muscle are very well developed, and it is unattached posteriorly. The teeth in the ascus are rolled up in a spiral. There is no pharyngeal pouch.
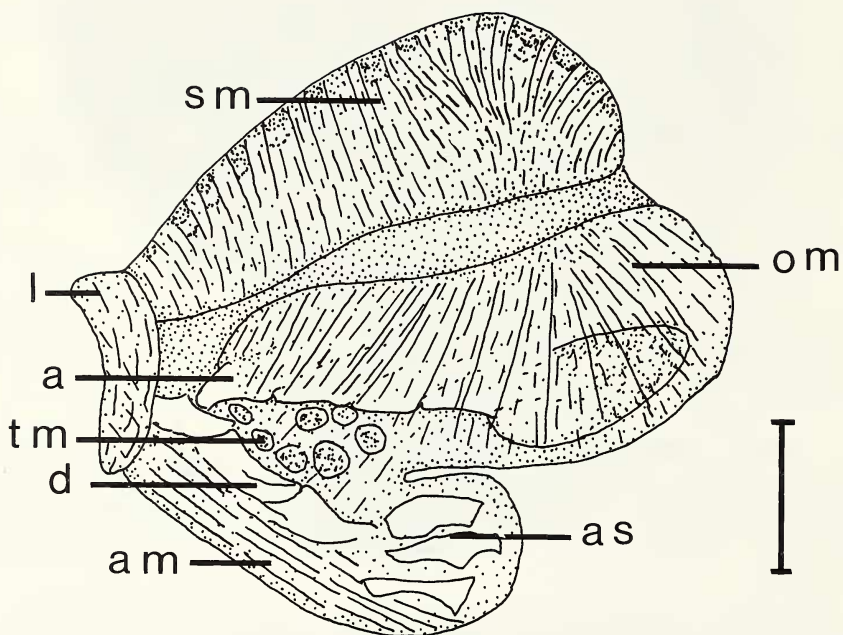
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Figure 5. Sagittally sectioned pharynx of *Ercolania translucens* from Rottnest Island, Western Australia. Scale line = 0.1 mm. Legend: **a** - ascending limb; **am** - ascus-muscle; **as** - ascus; **d** - descending limb; **l** - pharyngeal lips; ***om*** - odontophore muscles; **sm** - dorsal septate muscle; ***tm*** - transverse muscles.

Fig. 5 shows the pharynx of *Ercolania translucens* from Rottnest Island, Western Australia. It has a thick dorsal septate muscle. The odontophore is rather flat, and the ascending limb of the radula is located in the ventral part. Radula suspending muscles are indistinct. There is a distinct layer of transverse muscles separating the ascending and descending limbs of the radula. The ascus-muscle is very thick, and its posterior end is unattached. The teeth in the ascus form a densely packed heap. A pharyngeal pouch is absent.
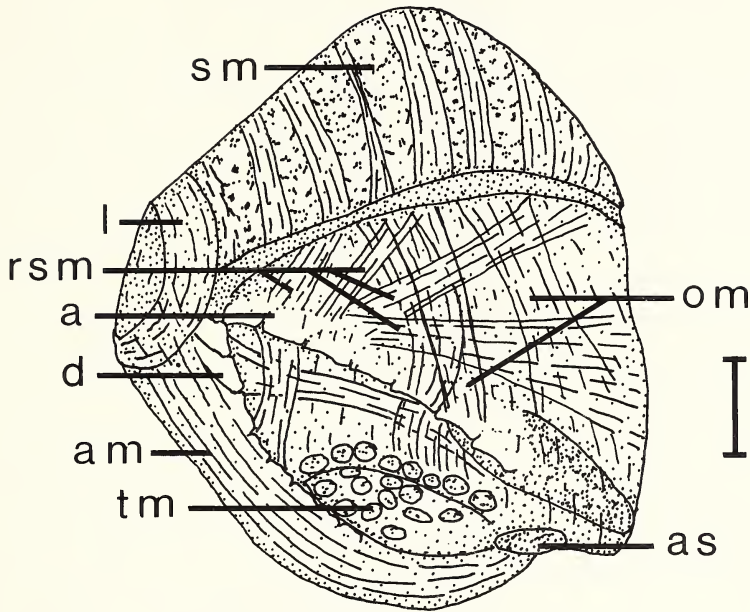
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Figure 6. Sagittally sectioned pharynx of *Elysia* cf. *maoria* from Rottnest Island, Western Australia. Scale line = 0.1 mm. Legend: **a** - ascending limb; **am** - ascus-muscle; **as** - ascus; **d** - descending limb; **l** - pharyngeal lips; **om** - odontophore muscles; **rsm** - radula suspending muscles; **sm** - dorsal septate muscle; **tm** - transverse muscles.

The pharynx of *Elysia* cf. *maoria* from Rottnest Island (Western Australia) has a tall, thick, domed dorsal septate muscle (Fig. 6). The dorsal and lateral corners of the pharyngeal cavity are pigmented. The odontophore is triangular in outline, and the ascending limb of the radula ascends diagonally from the postero-ventral edge of the pharynx to the antero-dorsal tip of the odontophore. The radula suspending muscles are prominent, especially dorsal to the ascending limb. The odontophore muscles are especially prominent dorsal to the ascending limb. There is a thick layer of transverse muscles separating the ascending and descending limbs of the radula. The ascus-muscle is long, almost as long as the pharnyx, thick and attached to the ventral surface of the pharynx over its entire length, and the thinwalled, densely packed ascus is external. There is no pharyngeal pouch.
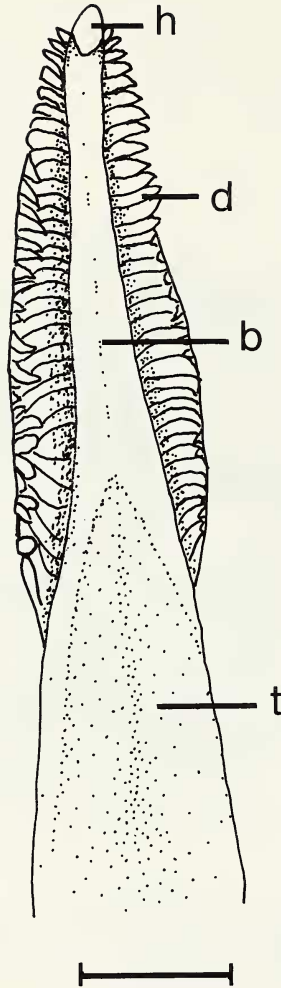
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Figure 7. Distal part of tooth of *Ascobulla fischeri* from Albany, Western Australia. Scale line = 10 μm. Legend: **b** - blade; **d** - denticles; **h** - hooked tip; **t** - triangular part of cusp.

RADULAR TEETH

The radular teeth of the Sacoglossa are large, articulating, and inter-locking. Only 1-3 teeth are exposed on the anterior, free surface of the odontophore (JENSEN, 1993a). They have a squarish base attached to the radular membrane. There are 3 basic shapes of the cusps of the radular teeth: teeth with triangular cusps with denticles along the lateral margins; blade-shaped teeth with lateral or median denticles, or completely smooth; and sabotshaped teeth with lateral denticles or smooth (JENSEN, 1991, 1993a).

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The teeth of *Ascobulla fischeri* (see SEM-photo in JENSEN & WELLS (1990)) have triangular cusps with long, thin lateral denticles. The denticles extend from the tip and slightly over half the length of the cusp. They originate from ridges along the dorsal edges of the cusp. The lower half of the cusp (Fig. 7) is broadly triangular. The distal part of the cusp is more blade-shaped, with a short, rounded blade. The tip of the cusp is hooked, and the hook fits into a depression on the back of the tooth in front. The base is short and stout, and there is a prominent articulation knob anteriorly (JENSEN & WELLS, 1990). The cusp is inclined about 35° to the longitudinal axis of the base.

In *Lobiger sagamiensis* the teeth are blade-shaped with lateral denticles (Fig. 8). The denticles are very short and only extend over part of the length of the cusp. The blade is narrow, and the tip of the teeth is bifid. The bifid tips, like the hooked ones, are used to interlock a long row of teeth. The bases are rather short, less than half the length of the cusp. The posterior articulation knobs are more prominent than the anterior one. The blade is 28° inclined to the base.

The teeth of *Cyerce antillensis* have a long, broad base and a rather narrow cusp with coarse denticles along the lateral margins of the distal part (see SEM-photo in JENSEN, 1993a). The lower part of the base has a broadly rounded median keel which fits into a cavity on the back of the preceding tooth. Distal to this cavity the back side of the cusp has a rather sharp edge. The articulation knobs anteriorly and posteriorly on the base are prominent. The cusp is only 18° inclined to the base. The teeth of *Caliphylla* are blade-shaped with a smooth edge (GASCOIGNE, 1979).

The teeth of *Costasiella pallida* (Fig. 9) are blade-shaped with smooth edges. The base is short with the most prominent articulation knobs posteriorly. The blade is long and thin, and the tip is slightly curved. The blade of *C. pallida* is inclined 23° to the base.

Hermaea cruciata has blade-shaped teeth with short lateral denticles (Fig. 10). The denticles are on low flanges which originate at the anterior edges of the base and run obliquely along the blade, almost to the tip. The blade is inclined 30° to the base. The base has prominent articulation knobs anteriorly and posteriorly, and it extends further back than the cusp. *Aplysiopsis smithi* has very peculiar looking radular teeth (Fig. 11). These teeth have been called sabot-shaped because of their resemblance to a Dutch wooden shoe (GASCOIGNE, 1977). These teeth have a very shallow base and broad cusps with a rounded tip and a prominent dorsal keel over which the tooth behind fits. In *Aplysiopsis* the teeth have denticulate margins. The base is short and the cusp is inclined 55° to the base.

The stiligerid genus *Ercolania* has sabot-shaped teeth. These teeth have a very shallow base, a conspicuous dorsal keel, and a deep cusp with flexible lateral edges. Articulation knobs are small or absent. In *E. nigra* the cusps have a short cutting edge dorsally on the tip of the cusp, and the tip is slightly hooked (Fig. 12).

Like most species of *Elysia*, *E. flavomacula* has bladeshaped teeth with median denticles (Fig. 13). The base is rather short with prominent articulation knobs anteriorly and posteriorly. There are no lateral flanges on the blade. The denticles are fine and have rounded edges. In *Elysia* cf. *maoria* the blade is relatively longer, and the denticles have pointed tips.
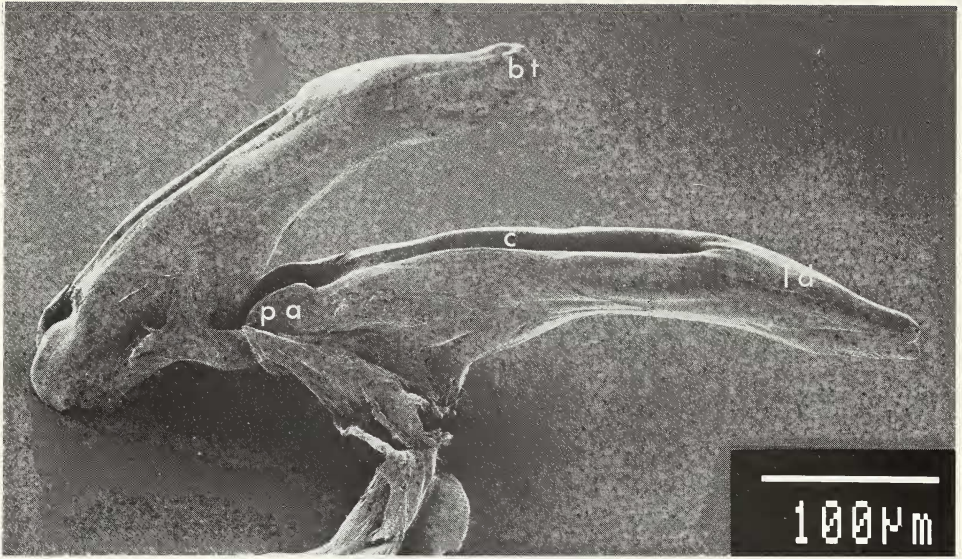
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Figure 8. SEM-photo of radular teeth of *Lobiger sagamiensis* from Hong Kong. Legend: **bt** - bifid tip; **c** - dorsal cavity; **ld** - latral denticles; **pa** - posterior articulation knob.
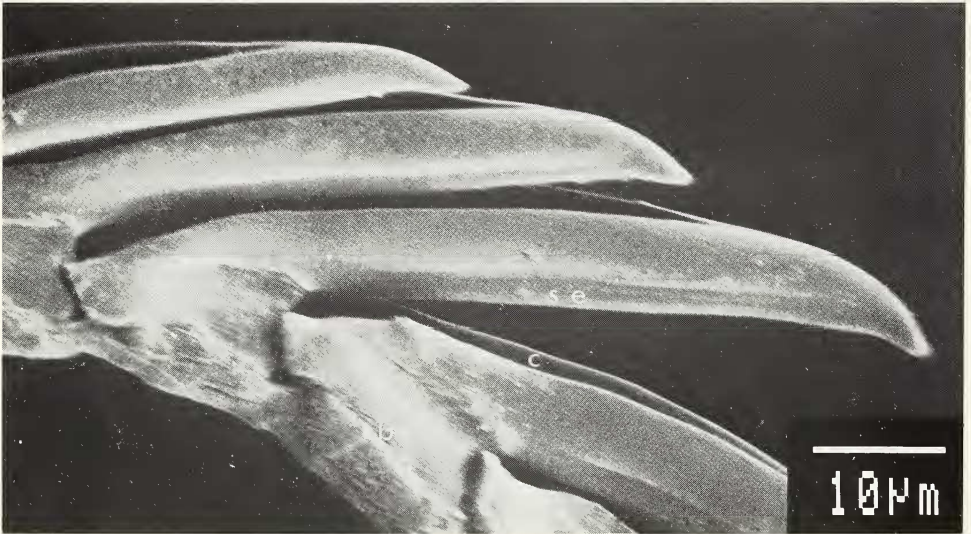

Figure 9. SEM-photo of radular teeth of *Costasiella pallida*. Legend: **b** - base; **c** - dorsal cavity; **se** - smooth edge.
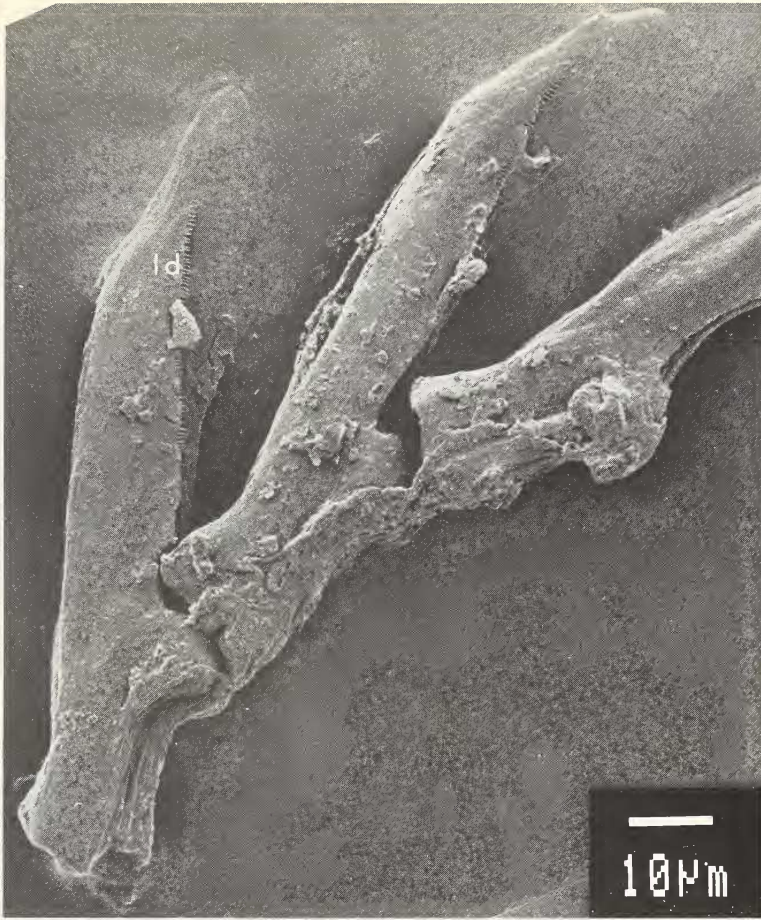
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Figure 10. SEM-photo of radular teeth of *Hermaea cruciata*. Legend: **ld** - lateral denticles.

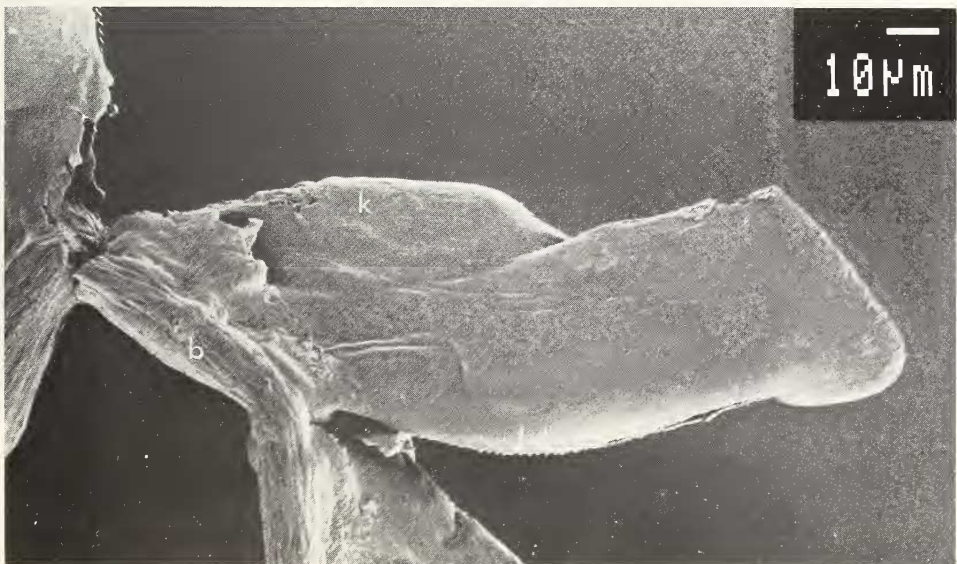Figure 11. SEM-photo of radular tooth of *Aplysiopsis smithi*. Legend: **b** - base; **k** - dorsal keel; **ld** - lateral denticles.
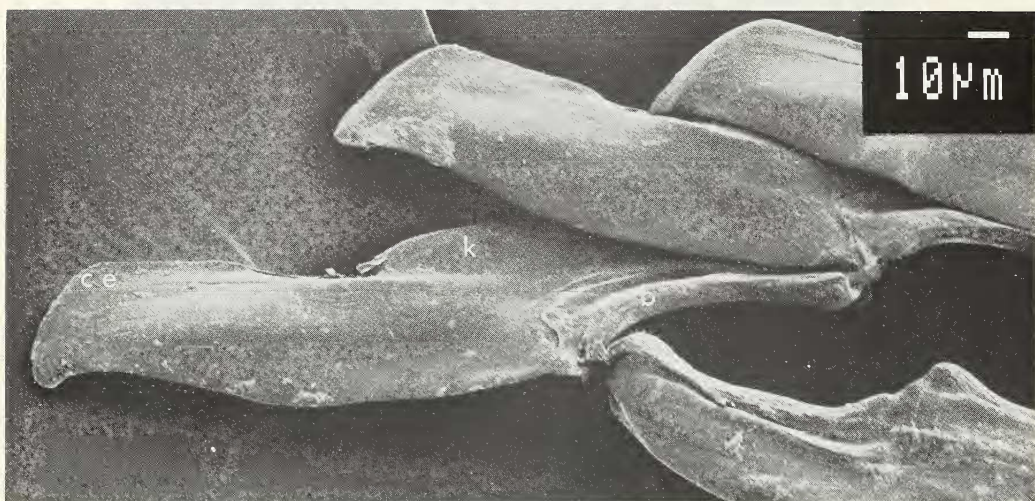
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Figure 12. SEM-photo of radular teeth of *Ercolania nigra*. Legend: **b** - base; **ce** - cutting edge; **k** - dorsal keel.
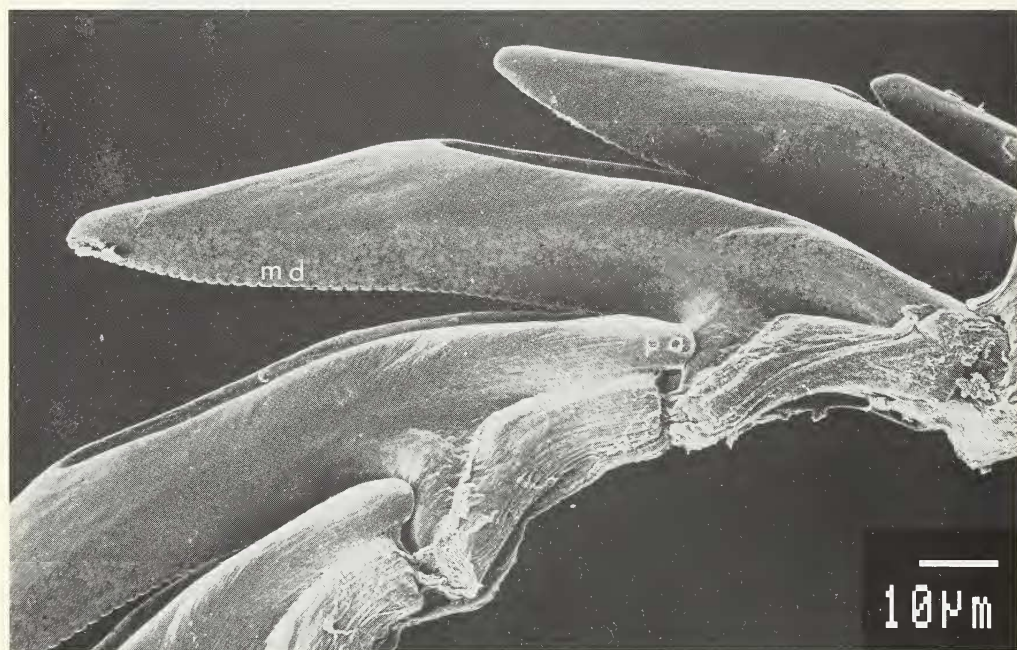

Figure 13. SEM-photo of radular teeth of *Elysia flavomacula*. Legend: **c** - dorsal cavity; **md** - median denticles; **pa** posterior articulation knob.

Discussion

The Sacoglossa have long been recognized as a well defined, monophyletic group. However, phylogenetic relationships within the group are not resolved (see f.ex. MARCUS (1982) and GASCOIGNE (1985)). Recently attempts have been made to describe characters which are useful for phylogenetic analysis (JENSEN & WELLS, 1990; JENSEN, 1991, 1992, 1993a,b in press).

Evolution within the Sacoglossa appears to be closely related to changes in diet (CLARK & BUSACCA, 1978; CLARK & DEFREESE, 1987; JENSEN, 1993a, in press). The shelled Sacoglossa all feed on algae of one single genus, *Caulerpa*. «Diet radiation» probably occurred at several stages of evolution. At all these stages species may have evolved which «reverted» to a more «ancestral» diet. Hence it is necessary to identify some morphological «innovations» in order to distinguish parallelisms from homologies.

The first step in diet radiation was from *Caulerpa* to other genera of the order Caulerpales (Fig. 14). These have similar cell wall structure, but often more complex thallus structure (JENSEN, 1993a). The second step involved a simplification in thallus structure, but a change in cell wall struc-
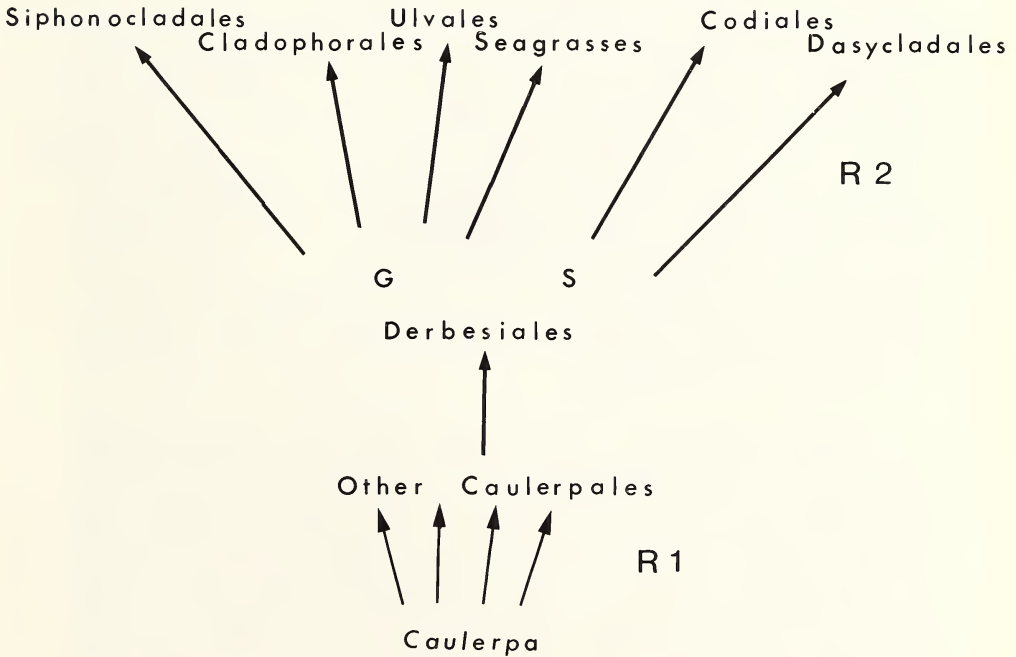
Figure 14. Diagram of diet radiation in the Sacoglossa. Abbreviations: **G** - gametophyte; **S** - sporophyte; **R1** and **R2** - first and second radiation.

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ture, namely the filamentous Derbesiales. These have heteromorphic life-cycles in which the gametophyte has xylan (as in the Caulerpales) and cellulose as the structural cell wall polysaccharides, and the sporophyte has mannan. Very few algal genera belong to this order, and this stage in sacoglossan evolution cannot be called a «radiation». However, after some species had adapted well to this food, the third step, which involved tremendous radiation (Fig. 14), could take place. At this stage some species chose to specialize on mannan cell walls and radiated to Codiales and Dasycladales; others specialized on cellulose cell walls and radiated to Siphonocladales, Cladophorales, Ulvales, or seagrasses. This may also be the stage from which diatom-feeding species and species feeding on red algae evolved.

The change from *Caulerpa* to other diets seems to have been associated with the loss of the shell. However, several species of *Elysia* and *Pattyclaya* feed on *Caulerpa*, and at least some species of *Polybranchia* are associated with this algal genus. Also, *Stiliger smaragdinus* feeds on *Caulerpa* (JENSEN, 1993b). Only thorough character analysis can resolve whether these species are morphologically «ancestral» or whether they have reverted to the «ancestral» diet.

CHARACTER ANALYSIS

1. *Dorsal septate muscle*: This is a composite muscle consisting of alternating layers of (semi) circular and radial muscles. The semicircular and radial muscle-bands work antagonistically, alternately constricting and widening the dorsal part of the pharyngeal cavity. In conjunction with the odontophore muscles, this functions as an efficient suction pump. The dorsal pharynx wall of most other gastropods is thin (FRETTER & GRAHAM, 1962), and the flat, indistinctly septate muscle of *Ascobulla* is considered plesiomorphic within the Sacoglossa. A flat dorsal septate muscle also occurs in *Volvatella*, *Lobiger* and *Thuridilla* (Fig. 15A) (JENSEN & WELLS, 1990; JENSEN, 1992, in press). In most non-shelled Sacoglossa the dorsal septate muscle is thick and domed (Fig. 15B), and in a few species, e.g. *Elysia australis* (Fig. 15C), the radial muscles converge in the central part of the pharyngeal cavity (JENSEN, 1992). This resembles the dorsal pharyngeal pump of some suctorial onchidorids (FORREST, 1953; CRAMPTON, 1977). These are apomorphic states.

2. *Shape of odontophore*: The odontophore takes up the major part of the pharyngeal cavity. Only the anteriormost tip is free. Anteriorly the odontophore is only attached ventrally. Further back the attachment broadens, and the odontophore is attached laterally as well as ventrally, leaving only a narrow, crescent-shaped pharyngeal cavity. In most gastropods the odontophore is located far back in a spacious pharyngeal cavity, and it is only attached to the pharynx wall ventrally (FRETTER & GRAHAM, 1962). In *Ascobulla* the odontophore is located rather far back, and this is considered plesiomorphic. Also, in most shelled Sacoglossa the tip of the odontophore is broadly rounded, and several teeth are exposed anteriorly. In most non-shelled sacoglossans the odontophore reaches all the way to the pharyngeal lips, and the tip is pointed. This is the apomorphic condition. In

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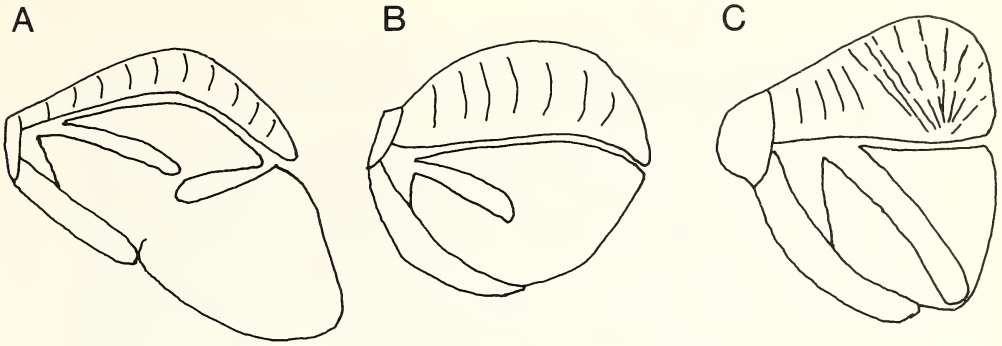
Figure 15. Schematic drawings of different types of pharynx. **A** - flat dorsal septate muscle, pharyngeal pouch, thin ascus-muscle, internal ascus, central ascendind limb. **B** - thick dorsal septate muscle, no pharyngeal pouch, external ascus, central ascending limb. **C** - thick, domed dorsal septate muscle, diagonal ascending limb, no pharyngeal pouch, thick, long ascus-muscle, external ascus.

Figure 16. Schematic drawing of radula suspension within odontophore. Legend: **drsm** - dorsal radula suspending muscles; **lo** - lateral attachment of odontophore; **lrsm** - lateral radula suspending muscles; **tm** - transverse muscles; **to** - dorsal surface of odontophore; **vrsm** - ventral radula suspending muscles.

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many sacoglossans the dorsal surface of the odontophore is rather flat. In others there is a distinctly pointed, tongue-like anterior part and a thick domed posterior part.
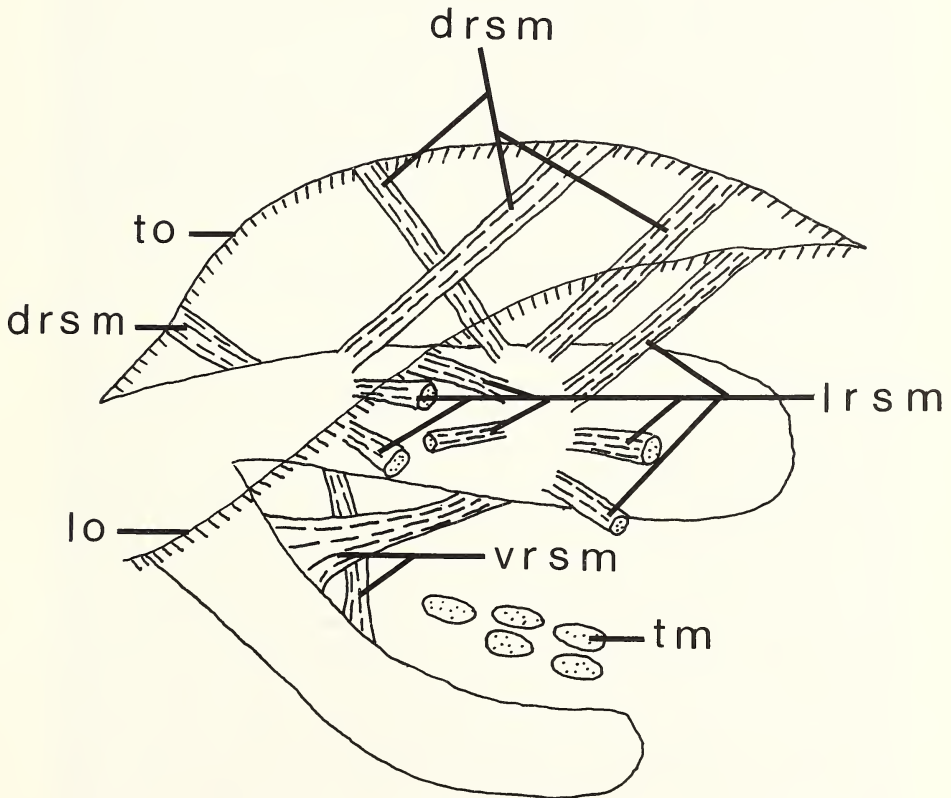
3. *Odontophore muscles*: The odontophore muscles are attached to the dorsal surface of the odontophore and extend to the ventral and lateral pharynx walls. In other gastropods the odontophore muscles are mostly associated with protraction and retraction of the odontophore (HURST, 1965). In the Sacoglossa the odontophore muscles function as a piston moving the dorsal surface of the odontophore up and down during the sucking phase of feeding (JENSEN, in press). Little variation is seen in the arrangement of the odontophore muscles, and function is probably the same throughout the order.

4. *Position of ascending limb*: The ascending limb of the radula is the equivalent of the radular sac in other gastropods. In the sacoglossans it is completely enclosed within the odontophore. In many shelled sacoglossans, e.g. *Ascobulla* and *Lobiger*, the ascending limb is located close to the dorsal surface of the odontophore (JENSEN, in press, present study). As the radular sac is external to the odontophore in most other gastropods (FRETTER & GRAHAM, 1962), this superficial position is considered plesiomorphic within the Sacoglossa. In many species of *Elysia* the ascending limb runs diagonally from the postero-ventral edge of the pharynx to the anterior tip of the odontophore (Figs 6, 15C), in others the ascending limb is located centrally within the odontophore (Fig. 15B). This is also seen in *Thuridilla* (Fig. 15A) (JENSEN, 1992) and many stiligeroids (JENSEN, 1993b). In *Aplysiopsis* the ascending limb is rather short, but steeply inclined within the odontophore. In *Ercolania* the ascending limb is located ventrally in the odontophore (JENSEN, in press, present study).

5. *Radula suspending muscles*: The ascending limb of the radula is attached by a complex system of radula suspending muscles, extending anteriorly and posteriorly in a fan-like manner to the dorsal, lateral, and ventral pharynx wall (Fig. 16). The radula suspending muscles run between the odontophore muscles, forming a dense network within the odontophore. This muscular attachment of the radular sac is a sacoglossan «innovation», and may have been instrumental to the development of suctorial feeding. Alternate contractions of posteriorly oriented and anteriorly oriented muscles move the ascending limb of the radula back and forth, and more differential contractions may allow very precise positioning of the leading tooth (=anteriormost tooth in descending limb). Few radula suspending muscles occur dorsal to the ascending limb in *Ascobulla*, and this is considered the plesiomorphic state. *Ercolania* and *Aplysiopsis* also have few dorsal radula suspending muscles. *Elysia*, *Thuridilla*, *Placobranchus* and *Caliphylla* have many dorsal radula suspending muscles (JENSEN, 1992, present study). These differences probably reflect differences in the function of the teeth.

The anteriormost teeth in the descending limb of the radula are the functional teeth. In many sacoglossans they are connected to the posterior end of the ascending limb by a strong radula suspending muscle. This muscle probably functions as a radula retractor muscle, but is clearly a part of the fanshaped arrangement of anteriorly oriented radula suspending muscles, and thus it is inappropriate to give a special name to this muscle. In some sacoglossans there is also a muscle connecting the anteriormost tooth of the ascending limb with some of the anterior teeth in the descending limb. This may correspond to a radula protractor or erector muscle (JENSEN, in press). In *Ascobulla* and *Lobiger* radula suspending muscles apparently attach to the descending limb throughout its lenght, and there are no transverse muscles forming a functional separation between the ascending and descending limbs of the radula (JENSEN, in press, present study).

6. *Transverse muscles*: In most sacoglossans there is a layer of transverse muscles forming a functional separation between the ascending and descending limbs of the radula. This allows independent movements of the two parts of the radula. The development of this thick layer is most likely one of the most important sacoglossan «innovations», and may have been responsabile for the ability to include algae other than *Caulerpa* in the diet. This layer is absent in the shelled sacoglossans studied so far, i.e. *Ascobulla* and *Lobiger* (JENSEN, in press, present study), and its absence is considered plesiomorphic. In many non-shelled sacoglossans, e.g. *Caliphylla*, the transverse muscles form a thick layer, which in transverse sections looks like a «stalk» attaching the ascus-muscle to the ventral surface of the pharynx (JENSEN, 1991).

7. *Ascus-muscle*: The ventral, longitudinal muscle-layer consists of densely set, parallel muscle-strands. Anteriorly the muscles extend from the lateral and ventral part of the pharyngeal lips. Anteriorly they form a rather thin layer around the ventral half of the pharynx. Behind the attachment of the odontophore the ascus-muscle concentrates on the ventral surface of the pharynx, surrounding the descending limb of the radula. In some species only the ventral and lateral surfaces of the descending limb are covered. This is seen mostly in the species having a pharyngeal pouch, and which have a weakly developed (or absent) transverse muscle layer. This is considered the plesiomorphic state. In the species having a thick layer of transverse muscles, the ascus-muscle surrounds the descending limb completely. This is an apomorphic condition. In the Elysiidae the ascus-muscle is long, in many species as long as the pharynx (JENSEN & WELLS, 1990; JENSEN, 1992). In most Stiligeroidea the ascus-muscle is short and the posterior end is not attached to the pharynx (JENSEN, 1993b, in press, present study). These two apomorphic states probably evolved independently. Physical separation of the ascus-muscle from the ventral surface of the pharynx also occurs in *Berthelinia* (JENSEN, 1993b). At the present time it seems most likely that this has occurred independently in the Stiligeroidea and *Berthelinia*.

8. *Shape of ascus*: In some sacoglossans the descending limb of the radula ends in a straight row with only the preradular tooth/ teeth bent over. This is seen in e.g. *Ascobulla, Volvatella, Pattyclaya* and some species of *Elysia* (JENSEN, 1992, present study). In other species the ascus teeth are rolled up in a spiral. This is seen in e.g. *Berthelinia, Caliphylla, Aplysiopsis* and *Thuridilla* (JENSEN, 1992, 1993b, present study). In other species the ascus teeth are densely packed in an irregular heap. This occurs in e.g. *Lobiger, Ercolania, Placobranchus* and most species of *Elysia* (JENSEN, 1992, in press, present study). As an ascus is not found in any other gastropods, the straight row is considered plesiomorphic, and the other 2 conditions are apomorphic. Both apomorphic conditions occur in shelled sacoglossans, hence it is not possible at the present time to determine whether one apomorphic state evolved from another, or whether both evolved from the plesiomorphic condition. Also, both apomorphic states occur in both major superfamilies of non-shelled sacoglossans. Thus they may have evolved several times independently.

9. *Position of ascus*: The ascus may be enclosed within the odontophore musculature as in *Ascobulla, Volvatella, Thuridilla* and *Placobranchus*, or it may be external as in *Lobiger, Caliphylla, Ercolania* and *Elysia* (JENSEN, 1992, in press, present study). This appears to depend on the extension of transverse muscles. The species with little or no transverse muscles usually have the internal ascus, and it is possible that in these species the ascus teeth form the functional separation between ascending and descending limbs. The internal ascus, then, is the plesiomorphic state.

10. *Pharyngeal pouch*: The pharyngeal pouch is almost solid muscle. In the species which have been examined till now, the lumen is paired, and the two narrow slits are separated by a thin epithelial membrane. The lumina may extend through the whole length of the pharyngeal pouch, or be restricted to the base of the pouch. In *Caliphylla* the lumina of the pharyngeal pouch are large (JENSEN, 1991, present study), and may have a different function than in the other species. The muscles of the pharyngeal pouch almost all have the same orientation, which is perpendicular to the longitudinal axis of the pouch. The pharyngeal pouch is usually attached lateral to the posterior end of the ascending limb. Hence the main function of the pouch musculature is probably to assist the ascending limb in piercing the algal cell wall. The musculature of the pouch probably functions in combination with the odontophore and radula suspending muscles, forming a muscular hydrostat (JENSEN, in press). Pharyngeal pouches occur in all shelled sacoglossans as well as the non-shelled family Polybranchiidae (=Caliphyllidae) (JENSEN, 1991), and also in a number of other non-shelled species, e.g. *Bosellia, Placobranchus, Thuridilla* and *Costasiella* (JENSEN, 1990, 1991, 1992). Postero-ventral pharyngeal pouches do not occur in other opisthobranchs, hence are a sacoglossan «innovation». A small, collar-like pouch as that of *Ascobulla, Oxynoe, Placobranchus* or *Costasiella*

must be considered plesiomorphic. Hence the pharyngeal pouch must have been lost several times within the Sacoglossa. It has apparently also evolved into large, «stalked» pouches more than once, e.g. *Lobiger* and *Polybranchia*/*Caliphylla*. Small, paired pharyngeal pouches occur only in the bivalved Juliidae.

11. *Shape of radular teeth*: Three basic shapes have been described previously (JENSEN, 1991, 1993a). The teeth of *Bosellia* are short and broad and have coarse lateral denticles (MARCUS, 1973). This is rather similar to the central tooth of many Cephalaspidea, and thus must be considered plesiomorphic. Triangular teeth with lateral denticles also occur in the shelled Volvatellidae, in most Polybranchiidae, and in the elysioid genera *Placobranchus*, *Thuridilla* and *Elysiella* (JENSEN, 1992, 1993a). However, numerous modifications have been observed. The triangular teeth of *Ascobulla* have a distal blade-shaped prominence, and in *Cyerce* there is a basal prominence (present study). Blade-shaped teeth occur in the largest number of species and apparently are not associated with any particular type of food (JENSEN, 1993a). They have most likely evolved independently at least 2 or maybe 3 times; in the Conchoidea (with lateral denticles), in the Elysioidea (with median denticles), and in the Stiligeroidea (smooth). In the blade-shaped teeth of the Oxynoidae and Juliidae the edge of the blade arises directly above the center of the anterior margin of the base (see SEM-photos in JENSEN & WELLS (1990) and JENSEN 1993a). In the Elysiidae the edge of the blade bends to one side, usually the left, of the base (see SEM-photos in JENSEN & WELLS (1990) and JENSEN (1992)). The blade-shaped teeth in the Stiligeridae are very similar to those of the Elysiidae, except that the inclination of the cusp to the base is usually much less than 20° in the Elysiidae and more than 20° in the Stiligeridae. The sabot-shaped teeth of *Aplysiopsis* differ in some important respects, i.e. inclination of cusp to base, relative length of base, and shape of tip, from those of the stiligerid genera *Ercolania*, *Limapontia* and *Alderia*. Sabot-shaped teeth occur almost exclusively in species feeding on algae having cellulose cell walls with micro-fibrillae in a crossed-fibrillar structure (JENSEN, 1993a). Thus they may have evolved in parallel in the Stiligeridae and the hermaeid genus *Aplysiopsis*. In *Alderia modesta* the base is somewhat broader than the cusp. At the base of the cusp is a small median crest (see SEM-photo in BLEAKNEY (1988)). The dorsal keel in rather shallow and does not extend all the way to the posterior end of the base. A few stiligerids, e.g. *Calliopaea* spp., and also the genus *Gascoignella* have teeth which do not fit into the above categories. The cusps form simple, smooth cones, like a chisel, and they have been called chisel-shaped (BABA & HAMATANI, 1970). These teeth may form a stage which is intermediate between triangular and blade-shaped, or between blade-shaped and sabot-shaped.

12. *Tooth denticulation*: The triangular teeth of *Ascobulla* and *Volvatella* have long lateral denticles on the distal part of the cusp. This is also seen in the polybranchiid *Cyerce antillensis* (JENSEN & WELLS, 1990; JENSEN, 1993a). In *Bosellia*, *Placobranchus*, *Thuridilla* and *Polybranchia* the coarse denticles extend over the whole length of the triangular cusp (MARCUS, 1973; JENSEN, 1992, 1993b). The teeth of *Elysiella pusilla* are peculiar in having denticles only along one edge of the narrow triangular teeth (see SEM-photos in JENSEN & WELLS (1990)). Also, the cavity of the cusp is very deep. Only the diatom-feeding *Elysia evelinae* and the seagrass-feeding *Elysia catulus* have smooth, triangular teeth. In the other seagrass-feeding species, *Elysia serca*, denticulation varies among populations (JENSEN, 1981, 1982, 1983, 1993a). Lateral denticles on the blade-shaped teeth of *Oxynoe*, *Berthelinia* and *Lobiger* originate from flanges near the dorsal side. Some species of *Elysia*, e.g. *E. timida* and *E. filicauda*, also have lateral flanges with small denticles (see SEM-photos in JENSEN & WELLS (1990) and JENSEN (1992)). However, these occur only on the distal part of the blade, and probably are not homologous to those of the shelled species. The lateral denticles in *Hermaea* are also on flanges, but these run obliquely on the blades from the anterior, basal part to the central, distal part (Fig. 10). In most species of *Elysia* the blade-shaped teeth have denticles along the median cutting edge (Fig. 13). A few elysiids, e.g. *Pattyclaya brycei* and *Elysia ornata*, have smooth, blade-shaped teeth (JENSEN & WELLS, 1990; JENSEN, 1992). Many stiligerids have blade-shaped teeth, and in these species the teeth are usually smooth, e.g. *Placida viridis*, *P. kingstoni*, *P. daguilarensis* (JENSEN, 1993a). Only a few species, e.g. *Stiliger smaragdinus* and *Placida dendritica* have median denticles (BLEAKNEY, 1989; JENSEN, 1993b). Smooth blade-shaped teeth also occur in the genera *Caliphylla* and *Costasiella* (Fig. 9). The sabot-shaped teeth of *Aplysiopsis* have denticulate edges, whereas those of the Stiligeridae have smooth edges. However, many species of *Ercolania* have one more or less prominent indentation on the lateral edges (JENSEN, 1985, 1993b).

13. *Tip of radular teeth*: In the Volvatellidae the tips of the teeth are hooked. This is used for interlocking the teeth in a row. Interlocking apparently is not important in *Cyerce*, *Bosellia* and *Placobranchus*; the tips are not hooked, and the dorsal cavities are shallow. In *Thuridilla* the tip of the tooth is slightly hooked, and the distal end of the dorsal cavity partly covers the tip of the tooth in front (JENSEN, 1992). In the species with blade-shaped teeth the dorsal cavity is deep and this locks the tip of the preceding tooth in place. In *Lobiger*, *Roburnella* and some species of *Berthelinia* the tip is bifid, and this further strengthens the interlocking of the teeth. In the species having sabot-shaped teeth, and also in some species of *Elysia*, e.g. *E. japonica* and *E. verrucosa*, having blade-shaped teeth, the tip is broadly rounded (JENSEN, 1985, present study). This appears to be correlated with diets of Cladophorales, i.e. cellulose cell walls with crossed-fibrillar texture (JENSEN, 1993a).

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14. *Articulation*: Posteriorly on the base are usually 2 articulation knobs which fit around the anterior base of the tooth behind. This prevents lateral dislocation of the row of teeth, which may be important when the radula suspending muscles work. Anteriorly is a more or less prominent articulation knob which probably is most important when the tooth pivots on the tip of the odontophore. Articulation knobs are very weakly developed in sabot-shaped teeth. The interlocking system in these species also prevents lateral displacement, and pivoting is probably not important in the function of these teeth. Weak articulation knobs are considered apomorphic.

Conclusions

The present study has shown that the buccal apparatus of the Sacoglossa contains several morphological «innovations» which are connected with diet radiation. Also, several structures have evolved in parallel in major lines of evolution in response to parallel changes in diet. The most important morphological «innovations» are the muscular suspension of the ascending limb of the radula within the odontophore, the development of transverse muscles between the ascending and descending limbs of the radula, blade-shaped teeth with median denticles, and sabot-shaped teeth.

Functionally the shift in importance from ascending to descending limb of the radula is a very important evolutionary event. In the species having pharyngeal pouches the ascus-muscle is usually weakly developed and there is little or no transverse muscles separating the ascending and descending limbs of the radula. In some species the ascending limb is also longer than the descending limb. Functionally the pharynx with pharyngeal pouches and weak separation between ascending and descending limbs of the radula is plesiomorphic within the Sacoglossa. Here the ascending limb is obviously most important in piercing the algal cell wall (JENSEN, in press). Many of these species have triangular teeth with lateral denticles. The «rasping» function of these teeth and the dietary limitations imposed by this method have been described previously (JENSEN, 1993a).

Loss of pharyngeal pouches has occurred in both major shell-less groups of Sacoglossa. This is associated with the development of transverse muscles to form a functional separation between ascending and descending limbs of the radula. Also, the ascus-muscle is very well-developed in these species, surrounding the descending limb of the radula completely.

In the Elysiidae loss of pharyngeal pouches was accompanied by a posterior elongation of the ascus-muscle (and descending limb of radula) to almost the entire length of the pharynx. In many species the ascending limb shifted to a diagonal position, leaving a thick muscle-layer above the radula posteriorly. Also, tooth shape changed from triangular with lateral denticles to blade-shaped with median denticles. Feeding method in these species is probably «cutting», i.e. the algal cell wall is pierced by the tip of the leading tooth and then slit open from the inside (JENSEN, 1993a).

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In the Stiligeroidea loss of pharyngeal pouches was preceded by the posterior separation of the ascus-muscle and descending limb of the radula from the ventral surface of the pharynx. In many species the ascending limb has moved to a ventral or anterior position (JENSEN, 1993b, present study). In some species the radular teeth are blade-shaped, usually with smooth edges, and feeding method is probably «cutting», as in the elysiids. The most advanced stiligeroids are the species with sabot-shaped teeth. These teeth function in a «piercing» manner (JENSEN, 1993a).

As pharyngeal pouches are found in several non-shelled species, in the Elysioidea as well as the Stiligeroidea, the shift in importance from ascending to descending limb must have occurred some time after the loss of the shell. Unfortunately it is not possible to link this shift with any particular change in diet. Except for *Polybranchia* the non-shelled species feeding on *Caulerpa* do not have pharyngeal pouches: they have blade-shaped teeth and distinct transverse muscles separating ascending and descending limbs of the radula. Hence they probably evolved from species which had a non-*Caulerpa* diet, and reverted to the ancestral diet.

Because the sacoglossan pharynx contains so many autapomorphies correlated with suctorial feeding, character analysis must include information on diets as well as functional aspects of feeding to identify parallelism and determine polarity of many characters. Although only a fraction of the known species have been examined in detail, it is hoped that the most important evolutionary changes have been identified in the present study.

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